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Distinct mutation accumulation rates among tissues determine the variation in cancer risk

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Cancer is believed to be a result of accumulated mutations. However, this concept has not been fully confirmed owing to the impossibility of tracking down the ancestral somatic cell. We sought to verify the concept by exploring the correlation between cancer risk and mutation accumulation among different tissues. We hypothesized that the detected mutations through bulk tumor sequencing are commonly shared in majority, if not all, of tumor cells and are therefore largely a reflection of the mutations accumulated in the ancestral cell that gives rise to tumor. We collected a comprehensive list of mutation frequencies revealed by bulk tumor sequencing, and investigated its correlation with cancer risk to mirror the correlation between mutation accumulation and cancer risk. This revealed an approximate 1:1 relationship between mutation frequency and cancer risk in 41 different cancer types based on the sequencing data of 5,542 patients. The correlation strongly suggests that variation in cancer risk among tissues is mainly attributable to distinct mutation accumulation rates. Moreover, the correlation establishes a baseline to evaluate the effect of non-mutagenic carcinogens on cancer risk. Finally, our mathematic modeling provides a reasonable explanation to reinforce that cancer risk is predominantly determined by the first rate-limiting mutation.

The variation in the number of mutations across different cancer types is widely noticed¹. Identification of these mutations is traditionally according to the genomic sequencing data of bulk tumors². Notable among the most frequently mutated cancers are basal cell carcinoma (BCC) and melanoma, which contain ~2,200 and ~800 mutations in the coding region^{3,4}. On the other side, some pediatric cancers such as rhabdoid cancer contain less than 10 mutations per tumor⁵. The variation is also seen across different cancers with similar involvement of environmental mutagens. For example, Glioblastoma multiforme (GBM) has ~5 times as many mutations as medulloblastoma^{6,7}. Interestingly, BCC, melanoma and GBM are among the common human cancers, whereas rhabdoid cancer and medulloblastoma are relatively rare, suggesting the hypothetical existence of a correlation between the mutation frequency in tumors and the cancer risk.

Meanwhile, the accumulation of mutations in somatic cells is hypothesized to be the fundamental reason for tumorigenesis¹. However, the correlation between mutation accumulation in somatic cells and cancer risk has never been worked out because of the technical limitation in obtaining the somatic mutation rate of any tissue⁸. A recent finding that cancer risk is correlated with the number of stem cell divisions highlights the hypothesis by suggesting that cancer risk is a result of accumulated genomic changes occurring by chance during DNA replication⁹. However, this study didn't take many common human cancers into account such as prostate cancer and breast cancer probably because of the lacking of data regarding the number of stem cell divisions in these tissues. Furthermore, solely attributing stem cell division to apparently higher rate of lung cancer in smokers versus non-smokers and of colorectal cancer in inherited mismatch repair deficiency patients versus normal colorectal cancer patients, is in against with the general realization that smoking and inherited mismatch repair deficiency increase the mutation rate without strong influence on the cell division¹⁰⁻¹². Therefore, there should be factors beyond stem cell divisions that contribute to mutation accumulation.

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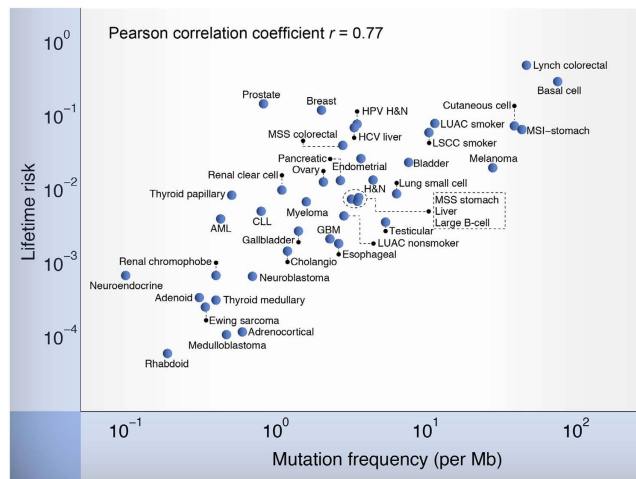


Figure 1. The correlation between the lifetime risk of cancer and the mutation frequency in tissue bulk of that cancer. Values and cancer names corresponding to the abbreviations in the figure are shown in Supp. Table S1.

The availability of the whole genome sequencing data of bulk tumor tissues¹³, however, presents an opportunity to evaluate the mutation rate of the ancestral somatic cell of each tumor. The accumulated mutations detected in bulk tumor sequencing outcompete the random mutations present in individual cells because the random mutations are masked by sequencing millions of cells simultaneously, as reflected by the undetectable mutation in normal control samples in cancer genomics studies^{6,14,15} but revealed in single cells¹⁶. Therefore, the mutations revealed by bulk tumor sequencing are largely a reflection of the mutations accumulated in the ancestral cell that gives rise to tumor (see Supplementary Text for more discussions). This supposition is strongly supported by the finding that half or more of the mutations detected in tumor bulk occur prior to tumor initiation, that is, during the growth of normal cells^{1,17}. Thus, investigating the correlation between mutation accumulation in tumor bulk and cancer risk can be an alternative way to mirror the correlation between mutation rate in somatic cells and cancer risk.

Results and Discussion

Correlation between cancer risk among tissues and mutation frequency in tumor bulk. First of all, the mutation frequencies of 41 different cancer types from the data of 5,542 human tumors detected by whole genome/exome sequencing were collected (Supplementary Text and Supplementary Table 1). Then the lifetime risk of cancers as a function of the mutation frequency of the corresponding cancers was plotted (Fig. 1). A strong correlation was observed between the two different parameters, with a Pearson correlation coefficient 0.72 ($p < 1.4 \times 10^{-7}$) in linear (x, y) coordinates and 0.77 ($p < 4.5 \times 10^{-9}$) in logarithmic ($\log(x)$, $\log(y)$) coordinates. The Spearman correlation is also significant (Spearman's $\rho = 0.75$; $p < 2.1 \times 10^{-8}$). This correlation strongly supports the hypothesis that there is statistically significant association between tumor mutation frequency and cancer risk and at least 50–60% of the variation of cancer risk is due to the difference of mutation frequency. To overcome the potential bias by using mixed data sources to estimate the mutation frequency, we selected 2,736 tumors across 29 cancer types that were sequenced using a uniform experimental pipeline and then analyzed using the same analytical pipeline from quality control, data processing and mutation calling¹³. This subset of data reveals a similar correlation between lifetime risk and mutation frequency (Supplementary Figure 1; $r = 0.66$, $p < 3 \times 10^{-5}$). A strong correlation was observed between the mutation frequency in this subset and the mutation frequency by our estimation ($r = 0.95$ in log-log scale, $p < 2.5 \times 10^{-14}$) suggesting our estimates of mutation frequency are highly robust against the data collection process from various sources.

This correlation applies to cancers across different tissues, associated with different environmental exposures and hereditary factors. For instance, when the mutation rate of the same cancer type is increased by mutagens (i.e., lung cancer patients as smokers vs. non-smokers) or hereditary defects (i.e., HNPCC vs. MSI-Colorectal cancer, MSS-stomach cancer vs. MSI-stomach cancer), the lifetime incidence rises in a corresponding rate. Another example is the most common type of human cancers – skin cancers including melanoma, squamous and basal cell carcinoma¹⁸, for which our result suggests that the differences in cancer incidence match the variation of mutation frequency. Importantly, the correlation is extremely robust even when the estimates of mutation frequency were allowed to vary significantly (see Methods).

Of noting is that the slope of the regression line in log-log scale is 1.09 (0.80–1.39, 95% CI) in Fig. 1 and 1.01 (0.55–1.46, 95% CI) in the subset with uniform experimental pipeline, indicating an approximate 1:1 relationship between mutation frequency and cancer risk. Under an ideal condition, if the mutations in bulk sequencing represent the mutations of the ancestral cell, both the mutations accumulated before its fundamental change toward preneoplastic growth and the first rate-limiting mutation to initiate the preneoplastic growth, are included. Therefore, the mutation frequency in our measurements should correlate with the frequency of mutation accumulation before the preneoplastic growth and the rate of the first rate-limiting mutation. We speculate that such a correlation between measured mutation frequency and the rate of the first rate-limiting mutation

that requires the release of more sequencing data and clinical data through the efforts of many cancer research cohorts.

Although in some cancers, epigenetic changes and other alteration types not covered in our analysis may represent the driving events of tumorigenesis²². For example, 13 recurrently epigenetic-silencing genes were recently reported by the TCGA project²³. However, these independent genomic alterations only reduce, not enhance, the correlation between somatic mutation and cancer incidence. Therefore, their existence could only highlight our conclusions revealed by the strong correlation we observed. In addition, our modeling provides a reasonable approximation of the results and is deliberately oversimplified comparing to the true complexity of tumorigenesis. Nevertheless, the discovery that cancer incidence is strongly correlated with mutation frequency in an approximate 1:1 ratio sheds new light to the predominant role of mutation on tumorigenesis and may have implications on understanding the cancer behavior. For example, our model provides a reasonable explanation of the excess relative risk of cancer incidence after exposure to radiation and the linear correlation between lung cancer incidence and smoking intensity (see Supplementary Text).

Methods

Tumor Samples and Cancer Risk. We included in our analyses a total of 41 different cancers from 5,542 samples obtained from 53 previous studies (Supplementary Table 2). All the mutation frequencies are based on results of whole genome sequencing (WGS) or whole exome sequencing (WES). The average mutation frequencies of most cancers were collected from literatures directly, or evaluated using the data from the literatures. We noticed that most of these datasets have been collected by the cbiportal database (<http://www.cbiportal.org>). Cancer types not included in this study were largely due to the lack of data or too few samples of that cancer type that were detected by WGS/WES.

When available, cancer lifetime incidences were obtained from Surveillance, Epidemiology and End Results (SEER) database (www.seer.cancer.gov)²⁴ and generated by their software DevCan²⁵, or obtained directly from a previous study⁹. If the data were not available this way, we using the epidemiological statistics to estimate the lifetime incidence for a specific cancer. Details of data collection and processing for each cancer subtype are provided in the Supplementary Text in separate sections.

Robustness Analysis. Mutation frequencies can vary markedly across patients within a cancer type^{1,13}, which may influence the robustness of our estimation of mutation frequency and the robustness of the correlation between mutation frequency and cancer risk. Therefore, we estimated the coefficient of variation (CV), defined as the ratio of standard deviation to the mean, of the mutation frequency by bootstrap. After bootstrapping the mutation data provided by the previous study¹³, we found that the CV of cancers was quite robust, and overall was about ~5%. Even for the melanoma and lung adenocarcinoma that mutation frequencies vary dramatically across patients, the CV is ~12.5% and ~5.6% respectively. Then, to allow the estimates of mutation frequency to vary significantly, we sampled from a normal distribution with a CV of 20% for each of the 41 cancer types. For example, in melanoma with a mutation frequency of ~28 per Mb, this allows a $(28 \cdot 0.2) \cdot (28 \cdot 0.2) \approx 31$ per Mb mutation frequency variation in either direction. We also perturbed the lifetime cancer incidence by using the variation level of annual incidence of that cancer using the SEER data during 2003–2012 based on the assumption that the variation of lifetime incidence should be lower than the variation of annual incidence. After 100,000 iterations, the average Pearson correlation was 0.703 ± 0.058 in linear (x, y) coordinates and 0.759 ± 0.014 in logarithmic (log(x), log(y)) coordinates. The average Spearman correlation was 0.734 ± 0.021 . The correlation was significant in all iterations, with highest p value of Pearson correlation < 0.05 in linear coordinates and $< 10^{-6}$ in logarithmic coordinates, and highest p value of Spearman correlation $< 10^{-5}$ (Supplementary Figure 3). All the statistical analyses were performed in MATLAB, version 2014a.

Mathematical Modeling. Based on the classic Armitage-Doll model²⁶, assume that for a progenitor cell evolving to a clinically meaningful tumor, the first rate-limiting step (driver mutation) and n ensuing independent steps are required, the cancer incidence can be given by

$$\log(I(t)) = \log(\mu t) + \log L p_1 p_2 \cdots p_n \frac{(t-1)^n}{n!}, \quad (1)$$

where μ represents the mutation rate per unit interval of time before the first rate-limiting step and $(p_1 \cdots p_n)$ represent the probability of ensuing steps $(p_1 \cdots p_n)$ per unit time interval during clonal evolution. Here, μt , given t is representative of lifetime, would be the accumulated mutation frequency in the ancestral somatic cell of tumor that contributes the majority of the mutations revealed by sequencing tumor bulk. This modeling provides a reasonable explanation of the 1:1 relationship between mutation frequency in tumor bulk and cancer incidence in log-log coordinates. More details of the mathematical modeling process are provided in the Supplementary Text.

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Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

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